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Review

Twenty years of postharvest biocontrol research: Is it time for a new paradigm?

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ABSTRACT

The use of biocontrol agents as an alternative to synthetic, chemical fungicides that are presently used to control postharvest pathogens, has many constraints and obstacles that make it difficult to implement their use as a practical control strategy. Over the last 20 years postharvest biocontrol research has evolved towards being more integrated into a production systems approach with greater awareness of industry concerns. More research, however, is needed in many aspects of the science and technology of postharvest biocontrol and in integrating biocontrol agents into combined pre- and postharvest production and handling systems. Better understanding of the mode of action of postharvest biocontrol agents, relationships between infection levels occurring in the field and development of postharvest decay, along with basic information on microbial ecology and survival mechanisms of biocontrol agents on fruit surfaces, is critical for the advancement of successful implementation of postharvest biocontrol technology. The past 20 years of postharvest biocontrol research has seen tremendous advances and the creation of several products. Nonetheless, numerous challenges and opportunities still exist as this field of research matures. This review is an attempt to examine the field of postharvest biocontrol as it has developed over the past 20 years, define the reasons that have limited its commercialization, and identify areas of research that need to be addressed if the potential of postharvest biocontrol is to be achieved. We have also introduced a new paradigm for biocontrol research that may provide new opportunities for increasing the efficacy and consistency of biocontrol products.

1. Introduction

In 1985, Wilson and Pusey published a featured article in which they presented their ideas on the potential of postharvest biocontrol and discussed their use of a strain of *Bacillus subtilis* to control brown rot on peach, caused by *Monilinia fructicola* (Wilson and Pusey, 1985). Prior to that publication, only one notable example of postharvest biocontrol, using *Trichoderma* to control *Botrytis* rot on strawberry (Tronsmo and Dennis, 1977), had been published. The seminal work by Wilson and Pusey (1985) provided the basic ideas and principles that, over the next 20 years, fostered a wealth of research and product development around the world (Wisniewski et al., 2007). So what has been accomplished in those 20 years? While in the early 1980s one could find 1–2 publications per year on postharvest biocontrol, now a literature search on the topic will bring up at least a hundred related publications per year, and over a thousand articles over the whole time period. Additionally, the development of numerous commercial products has been pursued with limited success. Without question, postharvest biocontrol has matured into a significant area of research. While Wilson and Wisniewski (1989) enumerated many of the first principles and concepts defining postharvest biocontrol research, and several reviews have been written over the years (Wilson and Wisniewski, 1994; Janisiewicz, 1998; Droby et al., 2000; Janisiewicz and Korsten, 2002; Droby et al., 2003; El Ghaouth et al., 2004; Palou et al., 2008), perhaps it is time to evaluate the progress that has been made and more importantly try to identify the challenges and ideas that will generate research and product development in the next two decades.

The original, primary justification for conducting postharvest biocontrol research was to reduce or replace the use of synthetic chemicals (Wilson and Wisniewski, 1989) because of concerns regarding their potential impact on human health (U.S. National Research Council, 1987), especially children's health (U.S. National Research Council, 1993), and the environment. The discovery of biotypes of postharvest pathogens that were resistant to the major postharvest fungicides, as well as the potential loss of registration for the use of some of fungicides, also added to the urgent need for alternative strategies. The assumption was that prospects for the success of postharvest biocontrol products were greater than

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those for biocontrol agents developed to manage soil and foliar diseases. Factors supporting this premise were the ability to better regulate the physical environment (temperature, humidity, etc.) during postharvest processing and storage, the ability to target high numbers of the biocontrol agent directly to the desired location of activity, and the overall value of the commodity. In practice, however, despite the advantages noted, the performance of postharvest biocontrol products is still subject to significant variability, which has limited their acceptance as a postharvest disease management strategy (Wisniewski et al., 2001, 2007; Droby and Lechter, 2004).

Currently, the use of chemical agents remains the major method of choice by far for managing postharvest rots and the few postharvest biocontrol products that are commercially available have limited use, mostly in niche markets. A survey of the literature also indicates that most researchers are using strains of a surprisingly limited number of yeast or bacterial species and most research has been limited to studying a new strain on a new commodity and/or perhaps against a new disease (Chand-Goyal and Spotts, 1997; Korsten et al., 1997; Leibinger et al., 1997; Teixido et al., 1998; Lima et al., 1999; El Ghaouth et al., 2000; Ippolito and Nigro, 2000; Janisiewicz et al., 2001; Jiang et al., 2001; Saligkarias et al., 2002; Karabulut et al., 2004; Larena et al., 2005; Zheng et al., 2005; Zhang et al., 2006, 2008; Guijaro et al., 2007; Sugar and Basile, 2008). It seems that the last 5 years have seen a tremendous amount of "reinventing the wheel" and little progress has been made towards wider commercial implementation of effective and economically viable biocontrol products.

At present there are only two commercial products available in the market for postharvest use, each having a very small market share of the technologies used to manage postharvest diseases. Biosave (*Pseudomonas syringae* Van Hall) registered in the USA and used mostly for the control of sweet potato and potato diseases (Stockwell and Stack, 2007), and "Shemer" (*Metschnikowia fructicola* Kurtzman & Droby) registered in Israel and used commercially for the control of sweet potato and carrot storage diseases (Kurtzman and Droby, 2001; Blachinsky et al., 2007). Two early yeast-based products, Aspire™ (Ecogen, US) and YieldPlus (Anchor Yeast, South Africa), are no longer available. BioNext (Belgium) and Leasaffre International (France) are developing a commercial product, based on the same yeast used in Aspire™, *Candida oleophila*, and a product based on the yeast, *Candida saitoana* is being developed by Neova Technologies (Abbotsford, British Columbia, Canada). In addition, a commercial formulation of *Candida sake* was recently developed and registered for use on pome fruit in Spain under the name "Candifruit". How these products will fare will largely depend on their ability to control postharvest diseases in a reliable, cost-effective and easy-to-use manner. This review is an attempt to examine the field of postharvest biocontrol as it has developed over the past 20 years, define the reasons that have limited its commercialization, and identify areas of research that need to be addressed if the potential of postharvest biocontrol, as first projected by Wilson and Pusey (1985) and Wilson and Wisniewski (1989), is to be achieved.

2. Basic principles of postharvest biocontrol research

2.1. Foundation of a research program

The identification, development, and commercialization of a biocontrol product is a long and costly process (Droby et al., 1998, 2000; Blachinsky et al., 2007) and therefore in the initial stages of a project it behooves the investigator to spend considerable time developing a "product concept" and try to anticipate any possible obstacles to commercialization. Wilson and Wisniewski (1989) described the criteria for an ideal antagonist (Table 1) and noted that special consideration was needed in identifying potential antagonists since, in the case of postharvest biocontrol agents, they would be applied to food. In this regard, they also noted that the potential of yeasts as postharvest biocontrol agents deserved special attention. In fact, the importance of yeasts has since been demonstrated, since the majority of postharvest biocontrol agents that have been reported in the literature and/or have been developed into products, are in fact yeasts. A diagram of the factors involved in the development of the products Shemer and Aspire™, based on the yeasts *M. fructicola* and *C. oleophila*, respectively, is presented in Fig. 1 and is relevant to the commercialization of biocontrol products in general. Among the important factors to consider are: (1) biosafety of the selected antagonist, (2) patent potential, (3) growth requirements and shelf life, (4) range of activity (commodities and pathogens), and (5) ease of use. If any of these factors are of concern, one may want to abandon further development despite the efficacy of the selected antagonist.

Table 1
Characteristics of an ideal postharvest antagonist for commercial development.

– Genetically stable
– Effective at low concentration
– Not fastidious in its nutrient requirements
– Ability to survive adverse environmental conditions
– Effective against a wide range of pathogens on different commodities
– Amenable to production on inexpensive growth media
– Amenable to formulation with a long shelf-life
– Easy to dispense
– Resistant to chemicals used in the postharvest environment
– Not detrimental to human health
– Compatible with commercial processing procedures

2.2. Isolation, screening, and identification of antagonists

The first step in developing biocontrol agents is the isolation and screening process which will largely influence the efficacy and ultimately its success under commercial conditions. For instance, Wilson et al. (1993) reported on their strategy to utilize fruit wounds to screen for potential yeast antagonists against postharvest rot organisms from unidentified microbial populations on fruit surfaces. This strategy allows for the rapid selection of a number of potential antagonists for the control of postharvest diseases of fruit with a minimal expenditure of time and expense and has been used in many postharvest biocontrol programs throughout the world. However, a shortcoming of this strategy is that it favors the selection of antagonists that are generally fast growers with the ability to colonize a specific niche (surface wounds) rich in nutrients, that mainly exhibit protective rather than curative activity, and appear to have little effect on latent infections (Droby et al., 1989; El Ghaouth et al., 2000). This may partially explain the lack of correlation between laboratory tests with host/parasite systems and performance of biocontrol agents/products under more varied commercial conditions (Droby et al., 1993, 2000; Wisniewski et al., 2001).

Since the method of screening will have a major impact on the type and properties of the antagonist that are identified, it is important perhaps to evaluate the consequence of the methods for screening that are presently being utilized and appraise whether or not they can be improved. As indicated, present methods largely favor the selection of microbial antagonists that have protective activity in small (3–5 mm deep) puncture wounds rather than curative activity or demonstrated activity in a wide array of wounds (bruises, scrapes, broken stems, broken epidermal hairs, etc.). In order for postharvest biocontrol agents to work under commercial conditions, perhaps screening methods need to better reflect the "real world" with potential antagonists being evaluated for both curative and protective activity, and screened on a wide array of wound types, as well as several different commodities

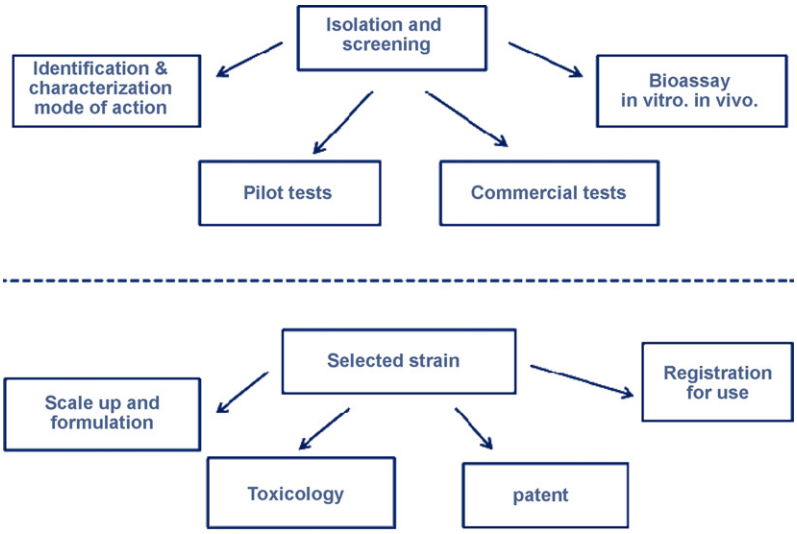

Fig. 1. Factors involved in the development of a biocontrol product.

and pathogens with different etiologies. Additionally, formulated preparations of the antagonist should also be evaluated as quickly as possible to determine if commercial methods of antagonist production have a major impact on biocontrol activity.

In addition to favoring the selection of organisms with protective activity, present screening methods also favor the selection of organisms whose primary mechanism of action is nutrient competition (Droby et al., 1989; Droby and Chalutz, 1994; Janisiewicz and Korsten, 2002; El Ghaouth et al., 2004; Wisniewski et al., 2007). This is despite the fact that a wide range of other potential mechanisms has been identified.

Perhaps a direct consequence of the type of screening procedures currently in use is the observation that several research programs in postharvest biocontrol worldwide have independently identified and selected antagonists from a very narrow range of species. While most researchers claim that their individual strains are superior to previously identified strains of the same or different species of antagonists, in reality most strains perform at nearly the same level of efficacy when tested independently and it has not been difficult to identify potential antagonists with commercial potential. The use of a variety of screening procedures would greatly increase the range of microbial species identified that exhibit biocontrol potential.

3. Mechanisms of action

While nutrient competition appears to play a major role in the biocontrol activity of many postharvest antagonists, it is rare for only one mechanism of action to be involved in suppressing a disease (Droby et al., 2000; Janisiewicz et al., 2000). A successful biocontrol agent is generally equipped with several attributes which often work in concert and may be crucial for controlling disease development. For example, colonization and nutrient competition may be related to the ability of biocontrol agents to adhere to specific sites, including both host and pathogen tissues (Wisniewski et al., 1991, 2007), exudation of specific enzymes (Castoria et al., 1997; Yehuda et al., 2003), the ability to induce resistance (Droby et al., 2002a), the ability to regulate population density at specific sites (McGuire, 2000), the secretion of antimicrobial substances (water soluble or volatile) and perhaps the production of specific active metabolites induced upon the interaction with fruit/plant tissues (Janisiewicz et al., 1991; Smilanick and Denis-Arrue, 1992; Schotsmans et al., 2008).

Information on the mechanisms of action for most of the antagonists investigated is still incomplete because of the difficulties associated with the study of complex interactions between a host, a pathogen, and an antagonist, as well as other resident microorganisms (Fig. 2). As illustrated, the performance of a biocontrol agent can be seen as the result of complex mutual interactions between all components (organisms). Although these interactions have been the subject of postharvest biocontrol research for 20 years, our understanding is still very incomplete. One of the more novel discoveries was the ability of some yeast antagonists to adhere to and parasitize pathogen hyphae (Wisniewski et al., 1991). This report was the first to document the ability of yeast to parasitize higher fungi. Other key factors that appear to play a role in the efficacy of yeast antagonists are the production of lytic enzymes by the yeast (Bar-Shimon et al., 2004; Friel et al., 2007) and their ability to tolerate high levels of salts (Wisniewski et al., 1995).

Castoria et al. (2003) demonstrated that the ability to tolerate high levels of reactive oxygen species (ROS) produced by fruit tissue is an essential characteristic of effective yeast antagonists. This discovery has raised many new questions about the role of ROS in biocontrol activity. Reports on the induction of resistance responses in fruit by application of antagonists within a wound or on the fruit surface has also been important in helping to understand the biology of postharvest biocontrol (Wilson and Wisniewski, 1994; Droby et al., 2002a,b; El Ghaouth et al., 2003). More recently, molecular approaches have been used to examine the role of glucanases in the biocontrol activity of the yeast *C. oleophila* (Yehuda et al., 2003) and to enhance biocontrol activity by overexpression of antimicrobial peptides (Wisniewski et al., 2003; Janisiewicz et al., 2008).

Two critical attributes of postharvest antagonists for which only rudimentary knowledge exists have to do with their ability to adhere to specific surfaces (pathogen, host and each other) and the ability to undergo fundamental changes in gene expression and metabolism when cell populations reach a specific level of density (quorum-sensing) or when they form a biofilm. A number of reports have illustrated the importance of quorum-sensing regulation in the formation of microbial biofilms (Parsek and Greenberg, 2005). Biofilm formation is a developmental process in which microorganisms form morphologically distinct multicellular structures, altered gene expression patterns, and enhanced resistance to stresses (Lazazzera, 2005; Nobile and Mitchell, 2005; Suntharalingam and Cvitkovitch, 2005; Visick and Fuqua, 2005). Environmental sensing and signal transduction pathways regulating morphogenetic transformations have been studied in depth in *Candida albicans*. Two

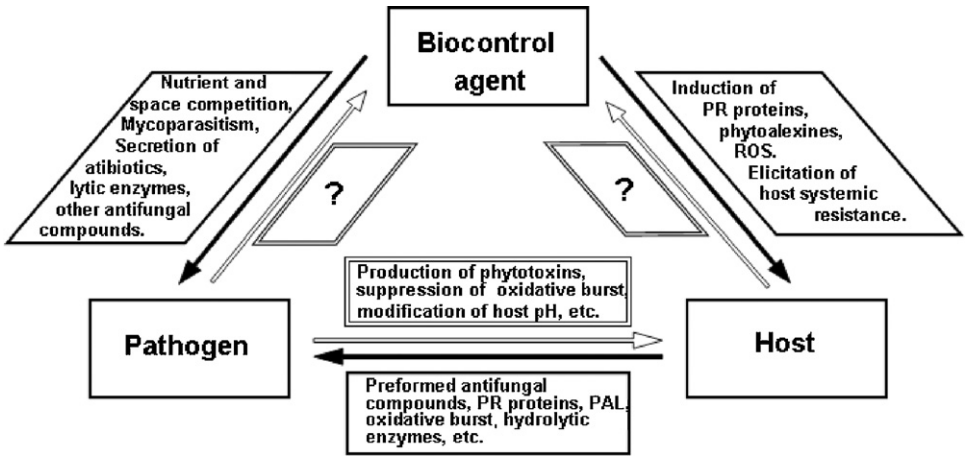

Fig. 2. Diagram of possible interactions between host, pathogen and antagonist. Question marks indicate interactions that have not been studied.

families of adhesin genes (*HWP1* and *ALS*) have been shown to play a critical role in host cell recognition, adhesion, invasion, and biofilm formation (Biswas et al., 2007). Furthermore, two quorum-sensing regulatory molecules, tyrosol and farnesol, coordinating phenotype switching in *C. albicans* (yeast-to-hypha and *vice versa*) have been identified (Hornby et al., 2001; Chen et al., 2004). However, little is known about the role of biofilms in the biocontrol activity of yeast antagonists used to manage postharvest diseases and mechanisms involved in its formation. We feel that understanding these mechanisms as well as the environmental cues regulating morphogenetic transformations in postharvest biocontrol agents will lead to the selection of more effective antagonists and new methods of optimizing their activity.

From a commercial point of view, complex modes of action mean that performance and efficacy might be much more dependent on production, formulation, packing, application, storage, etc. This highlights the need to develop rapid, reliable and economical methods of quality testing. Currently, apart from growth assays (which may not reflect biocontrol activity) the only tests available rely on testing the formulated product directly on fruit (commodity) assays. This can be a long process if conducted in a comprehensive manner and in the end it may not reflect performance under commercial conditions.

4. An expanded view of biological control

During the course of our research, we realized that if postharvest biocontrol was going to be commercially successful, a broader concept of biological control would be needed. Plant pathologists have mainly adopted the entomologists' definition of biocontrol, which involves the control of one organism with another organism. But, a plant disease is not an organism, it is a process. This process can be influenced at different levels: the pathogen, the micro-environment, and the host. For example, application of a biocontrol agent at a time that prevents establishment of the pathogen in the host tissue, given that the attachment of pathogen propagules to the host surfaces and the early stages of germination are critical to successful infection. The micro-environment (e.g. surface wounds) can also be altered to directly or indirectly affect the pathogen. The pH and nutritional composition of the infection site can be manipulated by the addition of salts, organic acids, or surfactants/adjuvants (Karabulut et al., 2001; Prusky et al., 2001; Porat et al., 2002; Conway et al., 2004, 2005; Qin et al., 2006; Hadas et al., 2007). Susceptibility of the commodity (host) may also be reduced by changing its physiology using various treatments to either slow down senescence or induce natural resistance responses (Lurie et al., 1995; Spadaro et al., 2004; Wan and Tian, 2005). These treatments include the use of plant growth regulators, ethylene inhibitors, modified atmosphere (MA) and controlled atmosphere (CA) and heat treatments. Also important to any disease management strategy is the reduction of inoculum through well-established cultural and management practices.

In accordance with a broader definition of biological control, in addition to the use of an organism, managing a plant disease could involve the use of a biological process or the product of a biological process. With this broader definition, a number of new approaches become available for developing effective, commercially successful biological control products and practices: (i) the classical (microbial antagonists), (ii) natural plant resistance, and (iii) natural antimicrobials which are the product of a biological process. While some of these broader approaches are being pursued by us and others to improve the efficacy of postharvest biocontrol agents, it is important to recognize that the above definition represents a paradigm shift in the concept of biological control and that this recognition may allow for a fundamental change in the way we think about biological control and the development of biocontrol products and strategies. It is a basic premise that paradigms drive scientific research and have a major impact on how we explore and interpret systems. This broader concept of biological control has been the driving force behind our development of a second generation of postharvest biocontrol products.

One new approach conceived using the broader definition of biological control, is based on the use of a combination of natural products and a yeast antagonist. Currently being commercialized by Neova Technologies (Abbotsford, British Columbia, Canada), these products consist mainly of a yeast antagonist, *C. saitoana*, and either chitosan (InovaCoat) or lysozyme (Inovacure). Natural compounds were shown to have strong direct effects on the pathogen (e.g. protective and eradicative activity) and affect the micro-environment and host resistance. The additives, when used in conjunction with a biocontrol agent, were found to enhance efficacy to levels equivalent to those found with available postharvest fungicides. Patents have been issued to cover this technology (El Ghaouth and Wilson, 2002; Wilson and El Ghaouth, 2002).

Another product, based on the use of a heat- and osmo-tolerant strain of *M. fructicola* (Kurtzman and Droby, 2001) and marketed under the trade name "Shemer" (Blachinsky et al., 2007), has taken the approach of preventing postharvest decay by administering several pre-harvest applications of a yeast to flowers and fruit in the field throughout the growing season. This approach addresses the problems of pre-established and latent infections. Shemer has been shown to be effective against rots caused by *Botrytis*, *Peni-*

cillium, *Rhizopus* and *Aspergillus* on strawberries (Karabulut et al., 2004), grapes, sweet potatoes, carrots and citrus (Blachinsky et al., 2007). Additionally, efficacy of the product for postharvest applications was markedly enhanced by addition of a relatively low concentration (0.1%) of potassium bicarbonate (Blachinsky et al., 2007). A similar approach of using multiple pre-harvest applications has been taken with the commercial product "Serenade" (AgraQuest, USA), labeled for use against both pre- and postharvest diseases (Marrone, 2002; Leelasuphakul et al., 2008). Based on *Bacillus subtilis*, the formulated product also contains fermentation metabolites. *B. subtilis* produces wide variety of secreted antimicrobial metabolites during growth and this feature was incorporated into the final formulated product which contains both live cells and metabolites obtained from the growth medium.

5. Commercial testing and industry perspective

The most critical criterion for the success of a biocontrol product is whether or not it performs effectively under commercial conditions, providing an acceptable and consistent level of control of the target disease/s. In most cases, as a part of the last phase of the commercial development process, biocontrol preparations are usually tested on their targeted crops at different locations using specific application methods. In order to conduct meaningful tests, large-scale production of a formulated biocontrol agent is required. These are costly trials to conduct and most often are done in association with a private company wishing to commercialize the biocontrol product.

It is essential that a formulated product, despite mass production of large quantities, retains the properties of the initial lab-grown cultures. The formulation must retain its species purity (not be contaminated) and the microbial cells must retain their genetic stability, cell viability, attributes as colonizers on fruit surfaces, as well as other aspects of their mechanism of action. Industrial fermentation is accomplished under conditions quite different from those in shake culture. The process must be cost-effective, rely on industrial by-products as nutrients and fermentation must be completed within 24–30 h (Hofstein et al., 1994). Downstream processing involves various steps, such as drying, addition of volume materials (inert ingredients), adhesives, emulsifiers and adjuvants. All these actions may adversely affect the properties of the selected biocontrol agent. The effect of commercial conditions on the physiological state of the biocontrol agent and its activity following rehydration is also critical. Various aspects of this topic were addressed in a series of articles by Abadias et al. (2000, 2001, 2003). Apart from these publications, no serious attempts have been made to address the large scale production and formulation technology of biocontrol agents. Information on the effect of industrial production practices as well as formulation technologies themselves should be investigated early in the development process before the product reaches costly commercial tests.

Results of tests performed under commercial or semi-commercial conditions with formulated biocontrol preparations indicate that inconsistency and variability in the level of disease control are among the most significant barriers preventing widespread implementation of biocontrol technology. In order to improve reliability and efficacy, efforts have been made to enhance efficacy and reliability by various means that include the addition of salts and organic acids (Droby et al., 1997; Karabulut et al., 2001), glucose analogs (El Ghaouth et al., 2000), food additives (Droby et al., 2002b; Karabulut et al., 2003; Qin et al., 2006) and integration with physical treatments (Porat et al., 2002; Zhang et al., 2006, 2008). Although promising additive and synergistic effects have been obtained, critical information on the interactions between antagonists, complementary treatments, pathogens, and commodities is still lacking. It is more than likely that each commodity–pathogen system has its own unique features and variables, so specific protocols will need to be developed and commercially evaluated.

Companies (including chemical companies) are always looking for new opportunities in the same or related markets as their existing products. If a biological product fits a market segment not occupied by a company's existing product line, then a biologically based product can be desirable product addition. An example is in the area of postharvest fruit disease control. Existing fungicides for postharvest disease control on citrus, pome fruit, and stone fruit have been reduced in number over the last decade because of regulatory restrictions and the development of pathogen resistance. The development of new synthetic fungicides for postharvest use is commercially unattractive because the registration process is costly and often not justified due to the small size of the market and the projected economic return. On the other hand, an effective biocontrol agent that does not have toxicity problems is relatively easier and much less expensive to register. So it seems that the market would favor the development of new biocontrol products. While this argument may sound convincing, in reality most major agrochemical companies are not interested in the postharvest market and the use of biological control agents because the market is too small. As a result, biocontrol products for the postharvest environment have been developed by small companies with limited capital or by companies that see the production of biocontrol products as an extension of their primary business.

6. Major obstacles

At present, small and startup companies have been the leaders in developing biocontrol products for postharvest use. However, the lack of financial resources and an established marketing network have posed major hurdles for small companies trying to commercialize their products. Since the size of the postharvest market is small, candidate organisms need to control a range of pathogens on a number of different commodities. This allows a company to market a single product to a wide range of producers and packing-house facilities.

Registration is required by regulatory agencies (e.g. Environmental Protection Agency (EPA), and European agencies) before any biocontrol agent can be used commercially. Although the registration process is not as expensive or time consuming as it is for synthetic chemical fungicides, this requirement must be taken into account during the development process. The registration package must contain a clean record of safety (for both humans and the environment) for the biocontrol agent, basic toxicological tests on the formulated product (eye and skin irritation, ingestion) and efficacy data including semi-commercial and commercial tests using relatively large quantities of fruit treated under conditions that resemble commercial practices. The registration of biocontrol products for postharvest use in the USA (through EPA) has been straight forward and several products have received registration. In Europe, however, the situation is more complex and until recently, registration has been difficult or impossible to obtain.

Concern has been raised about the health and safety of introducing antagonists into our diet. Although this may represent an obstacle to public acceptance of this technology, the majority of postharvest biocontrol agents were originally isolated from fruit and vegetables and are indigenous to agricultural commodities. Humans are exposed to them daily when consuming fresh vegetables and fruit. Even though these antagonists are introduced in large numbers to the surface of a commodity, they survive and grow only in very restricted sites on the fruit surface (e.g. surface wounds). After their introduction on intact fruit surfaces, antagonist populations usually diminish to the level of natural epiphytic microflora within a very short period of time.

Maintaining cell viability is fundamental in commercial formulations of biocontrol agents. Biocontrol products should have a shelf-life of at least 1 year at either room temperature or under refrigeration. As previously mentioned, in commercial formulations, the genetic stability and physiology of microbial antagonists may be compromised. Questions related to cell physiology and metabolism after rehydration are of utmost importance. Reports have demonstrated, for example, that conidia of *Trichoderma* spp. formulated in commercial products were significantly slower to germinate and colonize (Hjeljord et al., 2000). Reproducibility in the performance of a formulated antagonist is the most important requirement of a reliable product. Packaging technology as well as preventing contamination of the final product and the development of improved invert emulsions with high water retention is still a challenge. Quality assurance (QA) guidelines must be developed and considered as determinants for acceptability.

The biological control of postharvest diseases is viewed with caution and skepticism by many in the agricultural community. Unlike the control of tree, field crop or soil-borne diseases, successful commercial control of postharvest diseases of fruit and vegetables must be extremely efficient, in the range of 95–98%. As of today, such levels of control can be reliably reached by biofungicides only when supplemented with low levels of synthetic chemical fungicides.

7. Challenges for the future

Over the past 20 years, biocontrol research has evolved toward being more integrated into a production systems approach with more awareness of industry concerns. As noted in this review, more research is needed in many aspects of the science and technology of postharvest biocontrol and in integrating biocontrol agents into combined pre- and postharvest production and handling systems. For example, combining chemical and biological approaches has proven to be very effective in preventing postharvest diseases, and can be used to control mixed populations of fungicide-sensitive and fungicide-resistant pathogens (Lima et al., 2006). Such an approach could ideally be used in cropping systems where disease forecasting models are available (e.g. strawberry, grape). This may lead to restricting the use of chemical fungicides only to when conditions are conducive for disease development. In the future, development of control strategies based on a systems approach should be developed where predictive models, early detection techniques, biological methods, and cultural practices are adopted specifically to meet the requirements of each crop. Management of grey mould on kiwifruit in New Zealand using non-chemical methods has been a success story. Adoption of summer pruning to create a more open canopy, and the use of pre-harvest predictive models and postharvest curing has effectively reduced *Botrytis* losses (Michailides and Elmer, 2000).

Although several mechanisms of action have been suggested for postharvest biocontrol agents, a deeper understanding of the tritrophic interactions of plant tissue–pathogen–biocontrol agent is still needed (Fig. 2). In this regard, *Trichoderma atroviride* harboring multiple copies of a glucose oxidase-encoding gene from *A. niger* was able to produce H_2O_2 following induction by fungal pathogens (Brunner et al., 2005). This new trait gave transgenic *T. atroviride* the ability to exhibit a higher hyperparasitic activity against fungal pathogens and increased its capability to induce systemic disease resistance in plants.

Induced resistance has been postulated to be one of the mechanisms of action of postharvest biocontrol agents (Droby and Chalutz, 1994). However, information about elicitors/effectors of the antagonist involved and our ability to genetically and physiologically manipulate them is still lacking. Fundamental knowledge on the physiology, genetic traits and molecular basis of colonization, survival and differentiation of biocontrol agents on plant tissue is needed. Questions related to the effect of host physiology on biocontrol activity are also unresolved. More research effort is needed in order to address the need to lower the effective biomass and the inherent production costs of antagonistic microorganisms to be used in practical applications, and to enhance the efficacy of these beneficial microbes. Suitable formulations of these agents could play a crucial role in their effectiveness by increasing their dispersion and colonization on fruit skin, by prolonging their survival in practical conditions, and by enhancing the mechanisms of action underlying their biological activity.

After decades of research, questions regarding the relationship between infection levels occurring in the field and development of postharvest decay remain unanswered. Pre-harvest application of postharvest biocontrol agents has been employed as a strategy (Teixido et al., 1998; Ippolito and Nigro, 2000; Larena et al., 2005). However, to develop more efficient methods for controlling postharvest mold contamination it is essential to elucidate the relationship between infection of various plant parts in the field and postharvest incidence of disease.

A more thorough understanding of the microbial ecology of fruit surfaces will help us figure out which problems to work on, how to approach them, when and where to apply the biocontrol agent, and predict situations in which biocontrol would not be expected to work. Ecology and microecology in relation to biocontrol agents was reviewed by Nelson (2004). For example, the use of biosensors can provide information about the nutritional status of biocontrol agents on plant surfaces that can be used to enhance biological control. *Pseudomonas fluorescens* strain A506, which needs iron to form an antibiotic toxic to the fireblight pathogen, was transformed to act as a biosensor for iron (Weller et al., 2002; Temple et al., 2004). The transformed bacterium was able to colonize apple and pear flowers, but flowers, having an iron-limited environment, were inhospitable unless treated with iron in the form of FeEDDHA. Collins et al. (2002) examined the activity of *Bacillus subtilis* on leaves with and without 1% β-glucan and found that higher populations of vegetative cells were more likely to be present after fourteen days in the presence of 1% β-glucan and that populations were more aggregated without β-glucan. Thus, the distribution of the biocontrol agent on the leaf could be manipulated. The factors that determine the presence of a natural protective microflora on the surfaces of fruit and vegetables has not been fully explored. The existence of plant genes that influence the composition of the natural microflora has been suggested (Wilson, 2008). These "biocontrol genes" may favor the establishment of organisms that are antagonistic to plant pathogens. This implies that a portion of a plant's resistance to pathogens may be due to the native microflora and that the species composition of this microflora may be specifically determined by the genetic composition of the plant rather than just randomly.

Critical knowledge on adherence to surfaces, growth and regulation of biofilm formation antagonists is also needed. Recently, the ability to form biofilms on the inner surface of wounds was indicated as a possible mechanism of biocontrol (Scherm et al., 2003; Ortu et al., 2005). Experiments carried out with a strain of *Saccharomyces cerevisiae* capable of forming a biofilm in liquid culture, demonstrated its effectiveness against *Penicillium expansum*, the cause of blue mold on stored apple fruit. The activity of this biofilm-forming strain was tightly correlated with the morphological phase during which the cells were collected. Only yeast cells collected from the biofilm phase were effective in limiting pathogen growth, apparently being able to colonize the inner surface of artificial wounds with more efficiency (Ortu et al., 2005). Interestingly Giobbe et al. (2007) reported recently that a strain *of Pichia fermentans*, which controls brown rot on apple fruit, becomes a destructive pathogen when applied to peach fruit. On apple surfaces and within an apple wound the antagonist retained its yeast-like

shape whereas colonization of peach fruit tissue was always characterized by a transition from budding growth to pseudohyphal growth, suggesting that pseudohyphal growth plays a major role in governing the potential pathogenicity of *P. fermentans*.

8. Concluding remarks

The use of biocontrol agents as an alternative to the synthetic, chemical fungicides that are presently used to control postharvest pathogens has many constraints and obstacles that make it difficult to implement their use as a practical control strategy. The advances made and commercial products thus far developed, although limited, nevertheless represent promising possibilities. In most cases, however, even commercially available products still need to be fine-tuned and enhanced. A probable scenario is that the use of postharvest biocontrol and biological products in general will continue to increase slowly but will complement or be combined with low risk chemical fungicides, natural antimicrobial substances and other physical means. Biocontrol will greatly benefit from the rapid development of concepts, knowledge and methods in various fields of biotechnology. The availability of more efficient DNA-based methods have thus greatly facilitated the surveying and identification of candidate organisms, the elucidation of modes of action, and the monitoring of biocontrol agent fate and activity after application. These advances provide new possibilities for insights into ecological constraints and in addition can be used to generate valuable data for registration purposes. Developments in proteomics and functional genomics will enable us possibilities to determine and follow changes in the physiological status of biocontrol agents and the effect of environmental stress. Changes is such aspects as the expression of crucial "biocontrol" genes during mass production, formulation and storage, or in response to exposure and contact with host plant tissue after application, will also be avenues of future research that will greatly advance the science of biocontrol.

Although dependent on consumer acceptance and governmental policy, even greater advances are foreseen if commercial use of genetically modified organisms (GMOs) as biocontrol agents will became viable. In such an event the possibilities for designing effective biocontrol agents and/or enhancing the efficacy and activity spectrum of available biocontrol agents would be greatly increased. Enhancing the expression of crucial genes and/or combining genes from different biocontrol agents are two obvious possibilities. Importantly, there is still a wealth of opportunity for the discovery of new antagonists because only a small fraction of the earth's microflora has been identified and characterized.

The past 20 years of postharvest biocontrol research has seen tremendous advances and the creation of several products. Nonetheless, numerous challenges and opportunities still exist as this field of research matures. We have attempted to identify critical obstacles to commercial success and how these obstacles may be overcome. We have also introduced a new paradigm for biocontrol research that may provide a host of new opportunities for increasing the efficacy and consistency of biocontrol products. Lastly, we have attempted to identify the research problems that will stimulate and motivate the next generation of biocontrol scientists.

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